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Blood-Plates and their Relation to Coagulation

Physiology
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Numeration of Blood-Plates and the Relation of the Same to the Process of Coagulation

. . . BY . . .

HENRIETTA CALHOUN

THESIS

FOR THE

Degree of Bachelor of Science in Physiology

IN THE

COLLEGE OF SCIENCE

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Henrietta Calhoun

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OF Bachelor of Science

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1901
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Enumeration of the Blood-Plates and the Relation of the Same to the Process of Coagulation.

Everyone who investigates blood at the present time takes into account the third element of the blood, the blood-plates. Hayem, Laker, Bizzozero, Ebarth and Schimmelbusch, and others have all seen the plates in the blood vessels in normal circulating blood so that now they are recognized as a normal constituent of the blood.

The characteristic properties of the blood-plates are, first their stickiness, and second the rapidity and readiness with which they break down. Both of these qualities make it a matter of some difficulty to obtain a count that is accurate. To make such a count there are two things to be considered

1. a method.
2. a perfect preserving fluid.

Almost all the methods are variations of two principle ones,- the Laker method and the pipette method. The Laker method of obtaining an enumeration of the plates is by the use of two preparations, the first for an ordinary count of the red corpuscles, and the second for a count of the red corpuscles and plates within a given area in the blood counter.

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From the ratio obtained an absolute value for the plates is easily obtained. As Hayem[87] and Eberth and Schimmelbusch[6] have suggested this method gives a low count because many of the plates stick to the edge of the wound, but when the prick is made, and the blood is drawn through a drop of the preserving fluid this loss is reduced to the minimum. This objection applies even more to the pipette method as will be shown later. Laker[10] used the Thoma-Zeiss haemocytometer for making his counts of the red corpuscles and plates. The other method is the pipette method, and by it the count is obtained directly without a comparison with the other elements of the blood. The blood is drawn up to a chosen mark in the pipette as quickly as possible, then the preserving fluid is drawn up after it and the two are well mixed. The mixture is placed on a blood counter. The plates are counted. All the usual precautions connected with use of a Thoma-Zeiss apparatus are taken. There are two objections to this method besides the one of the plates lost around the edge of the wound; first, the plates adhere in large numbers to the sides of the pipette-tube; second,

+ The numbers correspond to the reference in the Bibliography

some time must elapse before the blood and the preserving fluid are mixed so there is a chance for the plates to become altered in form. I have had blood clot in warm weather before I could draw it up to the mark in the pipette, and my experiments (see page 26) show that the plates disappear when blood clots. Van Emden[17] has made a comparison of the two methods showing that the difference in count is within the limit of error. But his results can not be applied to the pipette method in general as he tries to do, because he worked with a cold pipette. Hayem, and Kemp, and others have shown that at a low temperature the plates do not readily break down. so that one of the errors of the pipette method as ordinarily used at room temperature is greatly reduced. Van Emden seems to realize this for he says one must be careful not to warm the pipette with the fingers. The Saker method is far more simple than the cold pipette as used by Van Emden. Muir[11] used the pipette method believing it was not accurate, but that he could obtain relative values which would be of use clinically. He tried the Saker method but abandoned it because he found it so difficult to get the proper proportions of the blood, and the

preserving fluid. I am convinced that this was not on account of a weakness in the method but rather on account of the preserving fluid that he used. He said with practice one could soon become so expert that only a few seconds would elapse before the blood and the preserving fluid were mixed. Hayem[8], Afanassiew[1], Prus[14], and Petroni[12] also used the pipette method. Determann[5] used the Gaker method putting the preserving fluid on the finger and drawing the blood directly into it; mixing well and farther diluting it, if necessary, on the cover glass. Gaker used the method as it has been already stated. Brodie and Russell[3], also used this method counting fields at random until 300 or 400 red corpuscles had been counted. Bizzozero[2], Fusari[7], and Pizzini[13] used the Gaker method, drawing the blood directly into a drop of the preservative. Kemp speaks of Gaker's method as the only practical method. Salvioli[16] took the blood drop from the freshly opened blood vessel of a Guinea pig on a paraffined glass-rod and quickly mixed it with the preserving fluid, then determined the ratio of the plates and red corpuscles. This is open to the objection that there is a chance for the plates to break

down while the drop is being transfered from the blood-vessel to the preserving fluid. Rabl[15] tried an entirely different method. He made dried preparations which he stained by a complex method and then he obtained his count by use of the ratios he obtained from these. Van Emden[17] has stated the obvious objections to this method, viz: the plates stick to the cover-glass just where they first touch it while the reds float by, and therefore the ratio of the plates to the red corpuscles in a given field is greater than it is in normal blood.

The question of a perfect preserving fluid is one of the utmost importance in obtaining an accurate count of the blood-plates. The fluid must be one that will absolutely prevent important changes in the plates, and yet a fluid which will keep the plates from sticking to the cover-glass; a fluid that will allow the plates to move freely, but which will also leave the red corpuscles in their normal condition. My first attempt was made with Bizzozero's fluid; .75% sodium chloride solution just tinged with methyl violet, then I tried methyl green as the stain. There was but a slight difference in the action of the two fluids; both preserved the

plates for a time but after twenty minutes the count had changed appreciably. Dekhuyzen[5] used this fluid as one of his preservatives and he says, "Nach einiger Zeit geht auch in der Conservirung-flüssigkeit die Form verloren." Weaker salt solutions do not exert a preserving effect, and stronger ones crenate the red corpuscles. I next tried another of Bizzozero's fluids: 14% solution of Na_2SO_4, but although it preserved the plates the haemoglobin of the red corpuscles was balled, and the balls were shed. They are easily confused with the plates. In all the mixtures of Na_2SO_4 in different per cents the haemoglobin balled. When the solution was too weak to affect the red corpuscles the plates were not preserved. Hayem's fluid:

Sublimate	0.5
Salt	1.
Na_2SO_4	5.
Water	200.

causes a precipitate (albumin?) to form which has an appearance not unlike that of a mass of broken down blood-plates. The field is not clear. I have tried various dilutions of Hayem's fluid but decided it was not convenient to use for making an accurate count. Sodium and potassium acetate in concentrated solutions preserve

the plates but the red corpuscles are badly distorted. In weaker solutions – one per cent – the red corpuscles are only slightly crenate, but the plates break down. Brodie and Russell recommend a glycerine solution:

NaCl 2% – 1 part

Glycerine saturated with Dahlia – 2 parts.

It preserves the plates well, and they move freely in the field but the two-thirds saturated solution of dahlia makes a medium whose color is too dense to count in easily, and it has the farther disadvantage which Brodie and Russell[3] admit, of decolorizing the red corpuscles in a short time. With Müller's fluid granules are formed in the field, and the plates are not easily seen. The red corpuscles are well preserved. Potassium bichromate solutions, 6%, 4% and 1%, gave beautiful clear fields at first, and the plates were well preserved, but in a short time granules appeared in the field. Determann[5] used a 1% NaCl solution with a 5% solution of potassium bichromate for some of his counts, but it is not a perfect preservative for any length of time. Van Emden[17] followed Petroni[12] and Prus[14] in using a modified Flemming's solution having the formula:

Osmic acid 1%	10 parts
Chromic acid 0,1%	10 parts
Glacial acetic acid	1 part.

With this solution the red corpuscles are destroyed. If Baker's method is employed with this fluid the ratio between the plates and the leucocytes must be used. This did not seem wise to me for two reasons, first, the leucocytes vary from time to time in the same day while the red corpuscles are more constant, second it is so much simpler to count the red corpuscles than it is to count the leucocytes, therefore I have not used this fluid at all. Osmic acid has been recommended as a preserving fluid and I hoped to find some solution of it which would answer my purpose. I tried various combinations of osmic acid with salt solutions starting from a pure 1% solution of osmic acid down to a .25% osmic acid solution in .75% sodium chloride solution. I found that it was a perfect preservative when the blood was well mixed with it in the proper proportions. The size of the drop of blood must be very small as compared to the size of the drop of the osmic solution. When there is too much blood the red corpuscles clump together, and the stronger the osmic acid solution the worse the

reds become clumped. Muir used an osmic acid solution with sodium sulphate and he said that the regulation of the proportion of blood and fluid was so difficult that the Laker method was not applicable clinically. This was not due to the method but to the fluid. Formaldehyde is well known for its preserving qualities and I made up first a 5% solution with 1% sodium chloride. The field was clear, the plates seemed very well preserved, but the red corpuscles were slightly clumped and swollen. A 2½% solution did away with this objection. The plates show clearly and are not altered, the red corpuscles are normal and the haemoglobin in them is unchange. The plates were able to move freely. For making permanent preparations I use both a 1% solution of osmic acid and a 5% solution of formaldehyde as a fixing agent. I prefer the formaldehyde solution because it preserves the haemoglobin in the red corpuscles, and because it does not fasten the red corpuscles as tightly on the cover-glass as the osmic acid does.

After the method and fluid have been decided on there still remains the stain to be considered. There are three requirements

for the stain viz:

1. It must stain the plates in dilute solutions.

2. It shall act on the plates quickly.

3. It must combine with the fluid used, and make a clear field.

Magenta and fuchsin in water solutions only stained the plates faintly. Bismarck Brown stains the plates but it is very difficult to keep the solution of formaldehyde that contains Bismarck Brown free from precipitate. For the best results with this stain it takes a fairly concentrated solution. Thionin destroys the red corpuscles. Congo red acts both as a stain and a preservative, but a precipitate is formed, and the field is not clear. Carmine does not stain the plates. Haematoxylin stains but it will not combine well with the solution of formaldehyde. Dahlia works well in saturated or half-saturated solutions but the color is so deep then that the field is not easy to work with. Methyl Violet, gentian Violet, Methyl green, and methylene Blue all stain the plates quickly in weak solutions. There is very little difference in them. I chose methyl green because it gave, I thought, a little clearer field.

All four of them combined well with a 2% solution of formaldehyde. Determann says that a saturated water solution of methyl violet is best. Muir advises the use of ~~to~~ saturated solutions of methyl blue and fuchsin in absolute alcohol as contrast stains for permanent preparations. With these the red corpuscles stain crimson, the leucocyte nuclei are blue, and the plates are violet. I found that simple haematoxlyn, Gentian Violet or Methyl Violet in water solutions made very satisfactory preparations. Tri-acid and aurantia-indulin-eosin stains were of no particular value in staining plate specimens. As far as I can decide there is no definite relation between the three elements of the blood as shown by their staining properties. The plates seem to be a distinct element by themselves. With haematoxylin, in very dilute solutions the plates stain faintly, the leucocyte nuclei stain deeply while the reds are not stained at all. The same is true of methyl violet and methyl green. Determann says "die Farbe der Plättchen gleicht der der Randzone derjenigen Leucocyten die keine starken Körner haben." I have been unable to reach any definite conclusions as to their structure or their origin from their reactions

with different staining reagents.

The fluid and stain with which I have worked is a solution made according to the following formula:

Formaldehyde 40%	4 c.c.
Distilled water	76 c.c.
Sodium chloride	2 gr.

Add enough of a solution of methyl green in 1% sodium chloride solution to give a destinct color to the fluid.

I found that a formaldehyde solution of 2% or 3% gave the most satisfactory results. The amount of sodium chloride may be increased to 6 grams. without materially altering the properties of the solution. When over six grams is added, however, the red corpuscles become crenate; the plates are not affected. There is one precaution to be taken when this fluid is used, viz: it must not be kept too long. After it is six weeks old one can not depend on it. In this fluid the plates appear as small flat discs of a light blue-green color, and they show a characteristic vibratory motion. They do not change position in the field but vibrate in the same place. The proportion of blood and formaldehyde does not need to be as carefully regulated as it does in the

case of osmic acid solutions for even when the blood drop is large the formaldehyde preserves the plates and the red corpuscles do not clump. Further dilution is easily made and the blood mixes readily with the preservative. In formaldehyde the plates are flat discs. I have seen them on edge, and rolling over in the fluid, but I have never noticed even the slightest tendency toward biconcavity. Hayem working with his fluid saw biconcave plates, and Kemp[16] has published photographs of the plates which show biconcave plates. Determann[5] says "Eine Dellenbildung habe ich nie gesehen". Van Emden[17] says that in Prus' fluid the plates appear, "als runde oder ellipsförmige opake Scheibchen – auf der Seite sind es nur feine Linien; öfters zeigen sie eine leicht oscillirende Bewegung. Die grössere Plättchen haben oft eine Wurstform, sind sehr deutlich feinkörnig, und zeigen vielfach ein excentrisch liegendes lichtbrechendes Flecken, wie im solches schon von Hayem beschrieben worden ist"

I have employed the Gaker method for obtaining an enumeration of the blood-plates. The finger was carefully cleaned and a prick was made with a spring lance. The number of red corpuscles was determined by means of a haema-

to crit. A drop of the formaldehyde solution was placed over the wound and a small drop of blood was drawn into it. This was then well mixed by means of a teasing needle, care being taken not to scrape epethelial cells off the skin. The drop was then droped on to a Thoma - Zeiss slide, and the cover-glass adjusted with the ordinary precautions. The number of blood-plates and red corpuscles in a certain field (from 16 to 48 small squares) were counted. The following equation gives the number of plates per. cu. mm.

$$R : P = r : x$$

In which:

R = number of red corpuscles in the field of blood counted.

P = number of plates in the field of blood counted.

r = number of red corpuscles per cu. mm. (obtained by haematocrit).

x = number of plates per cu. mm.

In the case of an operation on a dog a jet of blood was taken directly from an artery into a watch-glass filled with formaldehyde solution. My results come high as compared with those obtained by others but this is due, I believe, to the perfect preserving qualities.

of the formaldehyde. To show that Bizzozero's fluid did not preserve the plates well I made check counts of the same field in two different preparations with this result:

Experiment I.

Time	Number of red corpuscles in field.	Number of plates in field.
3:00 P.M.	99	13.
3:30 P.M.	99	11
4:00 P.M	95	10
12:30 P.M. the next day	92	5.

or in other words the ratio of plates to red blood corpuscles would have varied as follows:

Time	Ratio Plates : Reds.
At 3 P.M	1 : 7.7
½ hr. later	1 : 9.1
1 hr. later	1 : 9.5
2½ hrs. later	1 : 18.5

Experiment II

Time	Number of red corpuscles in field	Number of plates in field
9:30 A.M	46	5
12 M.	47	3.

or expressed in the ratios:

Time	Ratio Plates : Reds
9:30 A.M.	1 : 9.2
2½ hrs. later	1 : 15.7.

For the results of my counts of the plates see Tables I, II, III & IV.

Subject.	Date.	Time	Reds	Ratio	Plates	Remarks.
C.W.F. 4.	Nov. 20-'00	1:30 P.M.	4,800,000	5.64:1	852,000	two hours after lunch.
" " "	Dec. 13-'00	4:30 P.M.	5,000,000	5.7:1	877,500	
" " "	Jan. 15-'01	2:00 P.M.	5,000,000	6.4:1	782,000	
" " "	" 17-'01	2:00 P.M.	5,000,000	5.6:1	850,000	
" " "	Feb. 14-'01	1:00 P.M.	5,000,000	6.0:1	832,500	
" " "	" 19-'01	1:30 P.M.	5,000,000	6.1:1	822,000	
" " "	" 28-'01	2:00 P.M.	4,900,000	5.9:1	832,000	
" " "	Mch. 5-'01	1:00 P.M.	5,000,000	5.45:1	915,500	
" " "	" 12-'01	2:00 P.M.	5,000,000	6.0:1	832,500	
" " "	" 19-'01	4:30 P.M.	5,000,000	5.66:1	822,500	
" " "	" 26-'01	4:00 P.M.	5,000,000	6.30:1	789,500	
" " "	Apr. 3-'01	1:00 P.M.	5,000,000	5.66:1	822,500	
" " "	" 9-'01	4:00 P.M.	5,000,000	5.8:1	862,000	
" " "	" 16-'01	5:00 P.M.	5,000,000	6.33:1	789,000	
" " "	" 23-'01	4:00 P.M.	5,000,000	6.0:1	832,500	
	Averages		498000	5.89:1	833600	

Series of observations made on the same man showing normal variation.

Subject.	Date.	Time.	Reds.	Ratio.	Plates	Remarks.
Men A.C.F. (13)	Jan. 18.-'01	9:00A.M.	5,000,000	5.7 :1	870,000	
" " "	Mch. 6-'01	2:00P.M.	5,000,000	4.3 :1	1,175,000 +	Played tennis all morning.
E.P.C. (12)	Jan. 16-'01	5:00P.M.	4,500,000	5.5 :1	818,000	Not normal.
" " "	" 21-'01	11:00A.M.	4,700,000	7.1 :1	622,000	
E.F.B. (2)	Nov. 27.-'00	1:30P.M.	5,000,000	5.8 :1	862,000	
" " "	" 28-'00	1:30P.M.	5,000,000	5.9 :1	854,000	
" " "	Dec. 4-'00	2:30P.M.	5,000,000	6.0 :1	833,000	
F.B.	Mch. 25-'01	4:00P.M.	5,000,000	5.2 :1	937,500	
A.B. (1)	Dec. 10-'00	9:30A.M.	5,100,000	5.5 :1	927,300	
" "	Nov. 20-'00	10:30A.M.	4,800,000	6.0 :1	793,400	
I.H. (5)	Nov. 21-'00	11:30A.M.	5,000,000	5.9 :1	851,000	
C.H. (6)	Nov. 21.-'00	10:30A.M.	5,000,000	5.2 :1	961,500	
G.W.L.	Jan. 21.-'01	12:00 M.	4,800,000	6.1 :1	784,000	
C.S. (8)	Dec. 13.-'00	10:30A.M.	5,000,000	6.0 :1	822,000	
O.O.S. (9)	Nov. 19.-'00	11:30A.M.	4,800,000	6.0 :1	791,000	
	Averages.			566:1	750800	

+ Pizzini says "moderate exercise increases the number of plates.

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Subject.	Date.	Time.	Reds.	Ratio.	Plates.	Remarks.
Women I.	Nov. 16	11:30A.M.	4,500,000	5.40 : 1	833,000	
	Dec. 4.	1:30P.M.	4,500,000	6.20 : 1	730,000	
	Jan. 15.		4,300,000	5.9 : 1	728,000	
	Jan. 16.	4:00P.M.	4,300,000	5.5 : 1	747,500	
	Jan. 21.	1:00P.M.	4,500,000	5.5 : 1	818,000	
	Jan. 23.	1:00P.M.	4,500,000	5.4 : 1	833,000	
	Feb. 20.	4:00P.M.	4300000	5.6 : 1	768,000	
	Feb. 23.	4:00P.M.	4,300,000	5.5 : 1	782,000	
	Apr. 8	12:30P.M.	4,200,000	5.0 : 1	840,000	
		Averages		5.5 : 1	842000	

Subject.	Date.	Time.	Reds.	Ratio.	Plates.	Remarks.
Women. II.	Jan. 17-'01	2:00 P.M.	4,900,000	5.5 :1	781,000	
	Jan. 22-'01	2:00 P.M.	4,500,000	5.4 :1	833,000	
	Jan. 23-'01	11:00 A.M.	4,500,000	5.6 :1	803,000	
	Feb. 20-'01	3:00 P.M.	4,400,000	5.8 :1	754,900	Check count made with Thoma-Zeiss gave 4,450,000 reds.
	Feb. 21-'01	4:00 P.M.	4,400,000	5.6 :1	778,000	
	Mch. 26-'01	11:30 A.M.	4,300,000	6.0 :1	717,000	
	Mch. 27-'01	1:00 P.M.	4,300,000	5.6 :1	768,000	
	Apr. 9.-'01	5:00 P.M.	4,300,000	5.5 :1	813,500	
	Nov. 14-'00	10:30 A.M.	4,400,000	5.4 :1	778,000	
	Nov. 15-'00	9:30 A.M.	4,400,000	5.8 :1	758,000	
	Nov. 16-'00	10:30 A.M.	4,400,000	5.6 :1	778,000	
	Nov. 18-'00	9:30 A.M.	4,400 000	5.4 :1	758,000	
	Jan. 15-'01		4,500,000	5.6 :1	803,000	
	Jan. 16-'01	11:00 A.M.	4,500,000	5.6 :1	803000	
	Averages			5.6:1	780457	

Number of plates per cubic mil. obtained by other observers.

Observer	No. plates	Ratio	Fluid	Method	Reference.
Afanassiew	180000 to 300000		.6% NaCl .6% dried peptone in distilled H_2O, tinted with methyl violet and carbolic acid.	Pipette method.	Arch. f. Klin. Med. 1884 Vol 35, page 217.
Bizzozero	200000	1:20	1% osmic acid 1 pt tinted with methyl violet .7% NaCl 3 pt.	Laker Method	Zeit. f. wiss. Mik. 1892 Vol. 9, page 231.
Brodie & Russell.	635300	Aver. ratio of five counts 1:8.5	2% NaCl 1 pt glycerine sat. with Dahlia. 1 pt.	Laker Method	Journ. of Physiology Vol 21, '97 Page 390--395. also, Zeit. f. wiss Mik. 1897 Vol. 14, pag 392.
Cadet	290000				Etude Physiologique des Elements figures du sang et en Particulier des Haematoblastes Thèse de Paris 1881 Pg. 82.
Determann		1:18 1:30 aver= 1:22	9% NaCl tinted with Methyl Violet, and 1% in NaCl with 5% Potassium bichromate.	Laker Method	Deutsches Arch. f. Klin Med. 1898. Vol 61, pg 365-411. also Zeit f. wiss Mik. 1899. Vol 26 pg. 86.
Fusari	180000 to 250000		Equal parts of osmic acid 1% & NaCl .75% tinted with Methyl Violet. Later substituted a solution Na_2SO_4 sp gr. 1075 for .75% NaCl.	Laker Method	Arch per le Scienze Mediche 1887 Vol 10, pg 235
Hayem	185000 to 300000		NaCl 1 Na_2SO_4 5 Na_2SO_4 5 Sublimate .5 dist'd. water 200	Pipette Method.	Du Sang. Paris[?]

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Plate V

Observers	No. plates Ratio	Fluid	Method	Reference
Laker	more than 400000 in Guinea pigs blood.		Laker	Sitzber. d. K.K. Acad d. Wiss Wien 1886 Vol 93 Pag 62.
Muir	200000 to 250000	Na_2SO_4 (Spgr 1022) colored with methyl VIOLET.	Pipette.	Journal of Anat. & Physiol. 1891 Vol 25 pg 267.
Petrone		Osmic Acid 1% 10 pts Chromic Acid 1% 10 pts Glacial Acetic = 1 pt. (Originated by Prus.)	Pipette	Retarat im Centralblatt f. innere Medic. 1895, pg 633
Pizzini	300000 to 350000	Same fluid as Bizzozero.	Laker	Reforma Medica, 1894. Vol 2, No 32 and 33.
Prus	500 000	Same as Petrone	Pipette	Medycyna. 1886 No 39 & 40. abs. in Centralblatt f. Klin. Med. 1887 p 469
Rabl			Obtains the ratio in dried preparations	Wien Klin. Wochenschrift 1896 No. 46.
Van Ermden	245000	Same as Prus.	Cold pipette and compares with Laker.	Fortschrift d. Medicine 1898. Vol 16 pg 281-295. also 241-251.

Blood-Plates and Coagulation. It is generally granted that plates break down during coagulation. The majority of observers who have given special attention to the histology of coagulation believe there is a connection between the plates and coagulation. Bizzozero[2] says, "The principal role in coagulation falls to the blood-plates and not to the leucocytes". Both Hayem[8] and Bizzozero[2] have shown that reagents or conditions which retard the breaking down of the plates, retard to precisely the same extent the formation of fibrin; while reagents which preserve the plates prevent the formation altogether. This seems to show that there is some connection between clot formation and the breaking down of the plates. Kemp[9] confirmed these observations and arrived at the same conclusion. Any enumeration of the blood-plates in a field of clotted blood would be practically useless. The blood-plates are found clumped together in badly disintegrated masses. A new method, however, of obtaining reliable information on this subject was suggested by some experiments of Bizzozero[18] in which he drew a certain proportion of the blood (about 1/3)

defibrinated it; re-injected it; drew a fresh specimen, and so continued the process of defibrination and re-injection until the blood was deprived of all its fibrinogen and refused to clot. He found that after complete defibrination the plates were too few to count, (see Table VI which gives his results.) He studied their regeneration on successive days after the bleeding but he did not study the effect of each stage in the fractional defibrination on the blood-plates. A determination of the number of blood-plates at each stage of the fractional defibrination would necessarily give us valuable data on the relation of the plates to coagulation. A series of experiments was therefore made to supplement the observations of Bizzozero[18], taking up this important part of the field which, so far as I know has not yet been investigated. In these experiments both the disappearance of the plates during fractional defibrination, and their regeneration afterwards. For the results see tables VII, VIII, IX and X.

Exp. I. May 17, 1884. Chien robuste, campagnard, wt 17 kilo.

Date.	Red Corpuscles.	Plates.	Ratio	Remarks.
May 17.			P:R.	Fasting since day before.
Before Operation.	5,224,000	210640	1:24.8	
After 9 Defibrinations	5,833,000	4,667	1:1,280	
May 18.	5,800,000	19,333	1:300	To 1200 reds, he found 8 whites & 4 plates.
May 19	4,375,000	46,842	1:93.4	" 3456 " " " 38 " " 37 "
May 20	4,420,000	124,860	1:35.4	" 2089 " " " 7 " " 59 "
May 22	4861000	365,000	1:13.3.	N.B. Plates higher than at start.
Exp II.	July 15, 1890. Adult dog, - long hair. wt. = 5.75 kilos.			
July 15 Before op.	4,794,000	399,540	1:12	Blood ceased to coagulate after sixth defibrination.
July 15 8 Bleedings After op.		Too few to count.		
July 16	4,117,700	38,847	1:106	
July 17	4,166,700	88,655	1:47.	
July 18	2,723,400	106,390	1:35	
July 19	3,684,200	167,470	1:22	
July 20	3,804,400	211,400	1:18	
July 21	4516100	410,560	1:11	

Bizzozero's[18] Experiments.

Date	Leucocytes	Red Corpuscles	Ratio P : R	Plates	Remarks.
Apr. 10, '01.	10 000	4600 000.	1:10.1	461000	Before Defibrination.
Apr. 10, '01.	7600	3750 000.		0 (?)+	After Defibrination. Blood will not clot. Room was cold all night.
Apr. 11, '01.	29000	3750 000.	1 : 14.5	272000	Dog had chills.
Apr. 12, '01.	19000	3950 000.	1 : 9.3	408600	Blood clots readily.
Apr. 13, '01	21800	3950000	1 : 10.5	376200	Dog in good condition.
Apr. 15, '01	25000	4000000	1 : 11.3	352500	Dog fasted 60 hours. Wound suppurating.
Apr. 16, '01.	25400	4200000	1 : 10.3	406000	Dog had just been fed.
Apr. 17, 01	30000	4000 000	1 : 11	364000	Wound suppurating Blood clots readily.

Method of Bizzozero. Dog No. 1. (Small dog.)

Operation performed April 10 '01 at 4 PM. Duration of operation 1½ hr. Blood taken from left femoral artery and injected into left femoral vein Six Defibrinations made. Wound dressed with carbolized vaseline. Anesthetic-Chloroform at first then ether.

+ There were no well preserved plates in the field, but there was debris that might have been broken down plates.

Date	Leucocytes	Red Corpuscles	Ratio P:R	Plates	Remarks.
Apr. 30, '01.		4500000	1:9.6	4 470000	Impossible to count leucocytes because blood clots so readily.
May 3, '01	17000	3550000	1:11.5	306500	
May 4, '01		3500000	1:14.3	244000	
May 6, '01		3700000	1:14.0	266000	
May 8, '01	22000	4000000	1:12.5	321800	Just before operation, 9.30 A.M.
May 8, '01	18000	4000000	1:22.4	178000	After first defibrination, 11.00 A.M.
May 8, '01	15800	4000000	1:38	105200	After second defibrination 11:30 A.M.
May 8, '01	13600	4100000	1:58.2	70400	After third defibrination 12:15 P.M.

Dog No. 2. (Large) Dog died at 12:30 P.M.

Operated on May 8, '01. Operation begun at 10:00 A.M. Anesthetic, Ether.

Three defibrinations made. Blood reinjected at 43° C.

Urine taken two hours after death showed no peptone.

Heart examined immediately after death showed no thrombi.

Dog in bad condition. Covered with dog-sucking louse (Haematopinus piliferus Burm.) Intestines ulcerated.

Date	Leucocytes	Red Corpuscles	Ratio P : R	Plates	Remarks.
May 14, '01	21600	5 350 000	1 : 15.1	337000	Before Defibrination.
May 14, '01	16800	5 500000	1 : 29	185000	Leucocytes counted in defib blood. After first defibriniation (6:30) Time 6:30 P.M.
May 14, 01		5400 000	1 : 124.2	43500	After second defibrination. Time 6:45 P.M.
May 14, '01	8600	5300000		no plates.	After fourth defibrination. Time 7 P.M.
May 14, 01					Dog died during 5th defibrin.

Dog No 3. (Small pug)

No thrombi in heart after death

Date	Leucocytes	Red Corpuscles	Ratio P : R	Plates.	Remarks.
May 26 '01	16400	5400000	1:14.3	379000	
May 27, '01	10000	5100000	1:14.0	392000	
May 28 '01	8800	5150000	1:11.6	444000	Before defibrination, 4:20 P.M.
May 28 '01	5200	5500000	1:13.5	401500	After first defibrination, 5:06
May 28, '01	3600	5000000	1:35.5	141000	After third defibrination, 5:29 P.M.
May 28, '01	9600+	5000000	1:32	156300	After fourth defib. dog died. blood taken from Left Ventricle for count. Heart beating faintly.

Dog No 5. (Weight 10 Kilos)

Detail of Defibrination

Considerable Haemorrhage

(1) Drawn, 4:55, injected 5:06,

2. Drawn, 5:11, injected 5:20.

3. Drawn, 5:22, injected 5:29

4. Drawn, 5:31, injecte 5:37

5. Drawn, 5:46, dog died.

Amount of blood drawn 250 c.c.

Blood drawn from femoral artery and injected into juglar vein.

Post-mortem: no clot in heart. Lungs and stomach normal.

+

Conclusions.

1. The Laker method is the one that is best suited for making an accurate enumeration of the plates.

2. An appropriate diluting fluid is equally important.

3 The best preserving fluid in which to count the plates is a 2% to 3% solution of formaldehyde.

4. The ratio of plates to reds in normal people is an ave. ratio of 5.6 : 1.

5. The the difference in ratio of the plates between men and women is within the limit of error.

6. The plates may vary within wide limits from day to day in the same person.

7. The number of plates per cu. mm. is from 910,000 to 730,000.

8. There is a definite relation between the plates and the process of coagulation. After the blood has been defibrinated until practically no fibrin is formed there are no plates found.

9. The number of plates diminishes rapidly with each step of the fractional defibrination.

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